TOX_SCREEN MANUAL

1 Common Interface

Please take into account that the program fields cannot be filled with any values, as well as some input parameters can be changed in certain limits only, otherwise a program error occurs. This is due to both a limitation defined by the program code and an inherent limitation inside the mathematical model itself. During its work, the application generates result images and saves their data into database. That process may last for several minutes. When server will publish images, the server will allow client to download all images from the right panel of icons or data in excel format.

The login page with the link to web-tool can be found using web-link https://www.biodevicesystems.com/panbiora. The page contains two manuals that can be downloaded by user: 1) Description on how models in the module “HEPATOTOXICITY” works and 2) Description on how models in the module “TOX-SCREEN” works. Clicking on the corresponding button the word file can be downloaded from the desktop. There are also two buttons with web-link to these modules are presented on the page. The user has an option to choose one of them to go on web-page of each module (**Figure 1**).

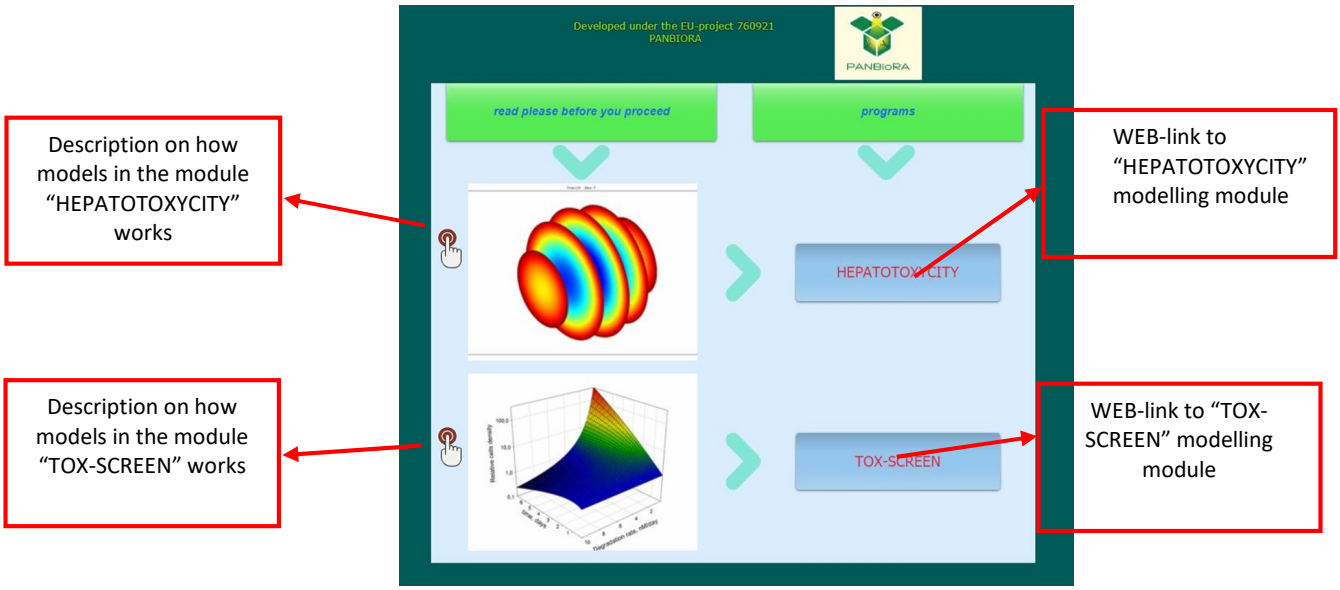


Figure 1. The login page with link to Web-tool.

The module “HEPATOTOXICITY” contains web-links to 3 microservices which can be used to start 3 programs: Model of cyto- (hepato-) toxicity under direct toxin influence; Model of cyto- (hepato-) toxicity under secondary (metabolite) toxin influence; Model of cyto- (hepato-) toxicity depends on toxin degradation rate.
The module “TOX-SCREEN” contains web-links to the main interface page of screening model page and can be used to start 6 programs (**Figure 1)**: Integrated screening model of cytotoxicity; Model of immune response to biomaterial (Adapted immunologic model); Microbiota model; Models of discriminative analysis of proliferation kinetics; Initial Model of cell death depends on toxin release from a biomaterial; Simplified hepatotoxicity screening model.

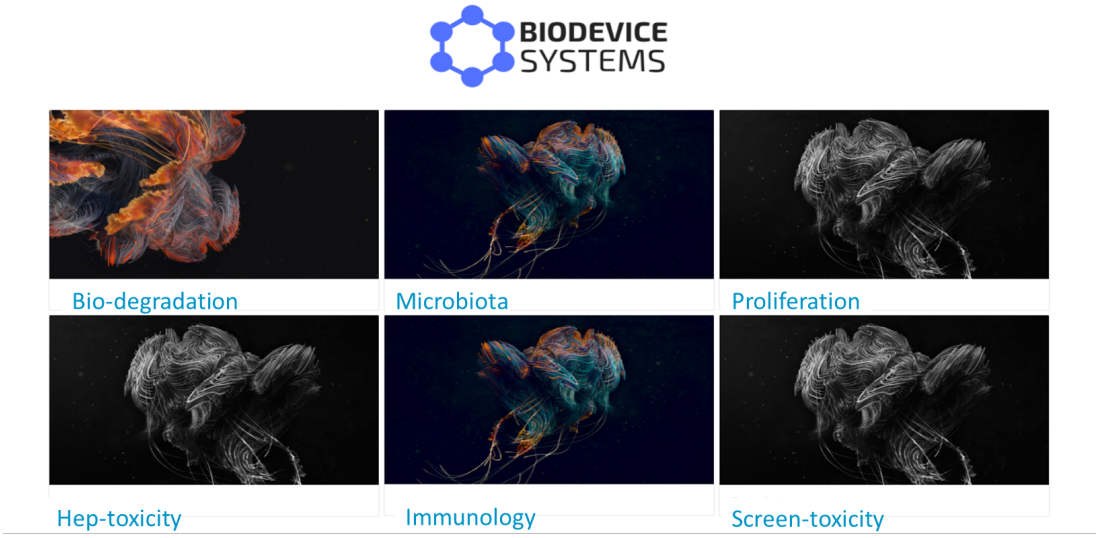


Figure 2. The main interface page of Module “TOX-SCREEN”

Registration of new user is available for the microservices, which are expected for frequent visits of end-users with screening assessment of biomaterials. Clicking on blue button on the upper-right axis of the desktop, user will open a registration page that will allow to set a password taking user` e-mail as a login (**Figure 3)**.

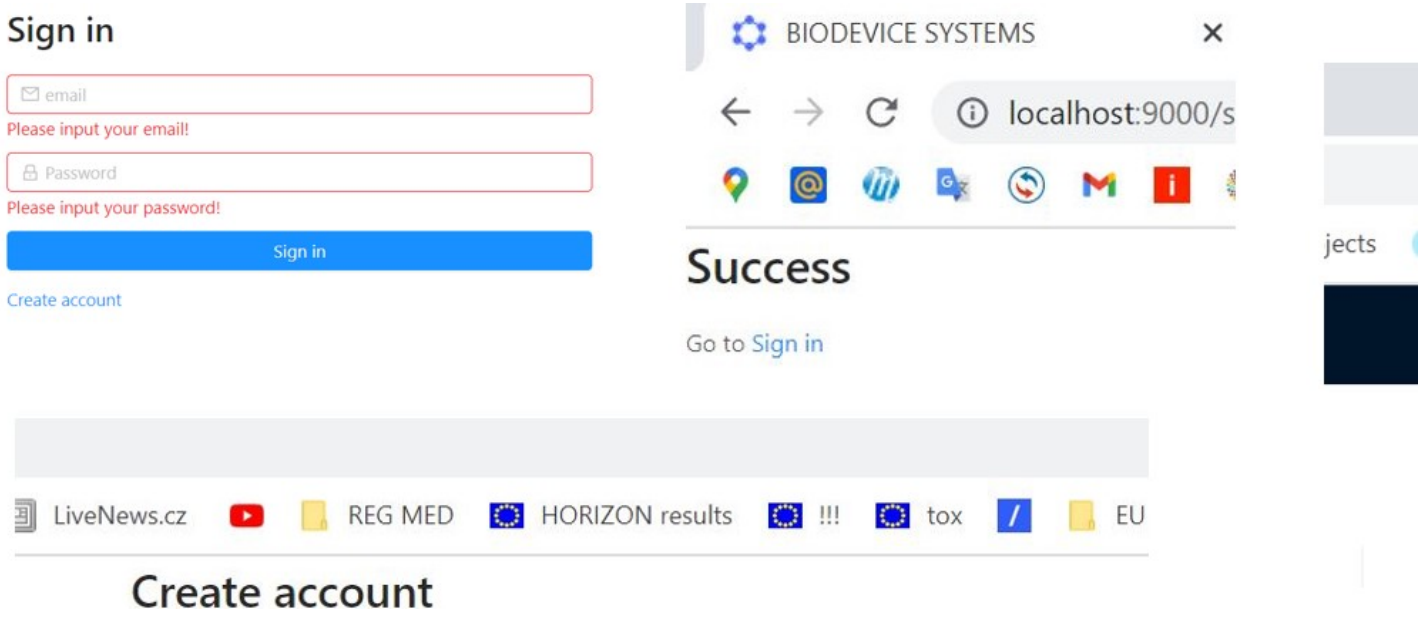
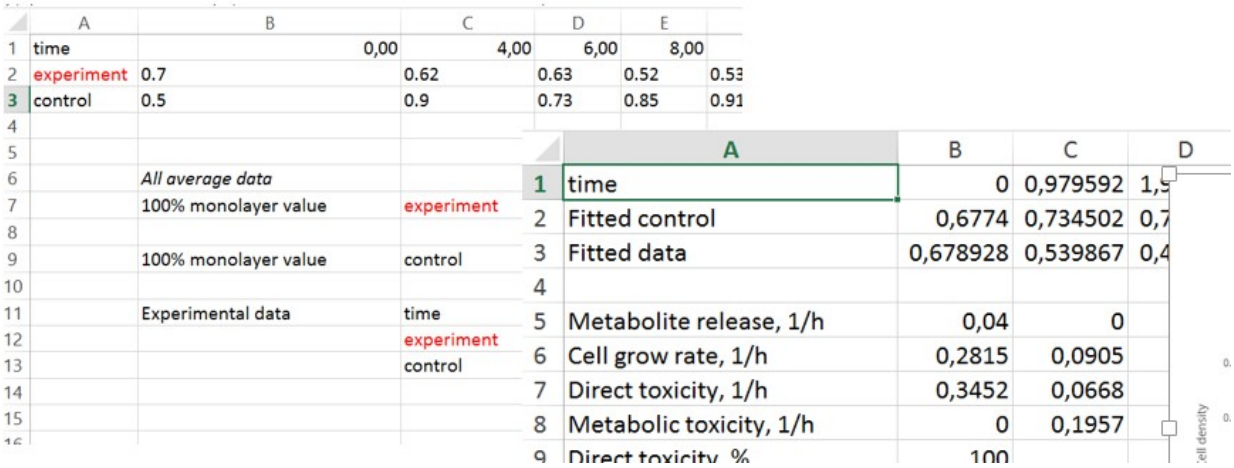


Figure 2. Autorisation form and plot-panel



	A	B	C	D	E
1	time	0,00	4,00	6,00	8,00
2	experiment	0.7	0.62	0.63	0.52	0.53
3	control	0.5	0.9	0.73	0.85	0.91
4						
5						
6		*All average data*				
7		100% monolayer value	experiment			
8						
9		100% monolayer value	control			
10						
11		Experimental data	time			
12			experiment			
13			control			
14						
15						

	A	B	C	D
1	time	0	0,979592	1,5
2	Fitted control	0,6774	0,734502	0,7
3	Fitted data	0,678928	0,539867	0,4
4				
5	Metabolite release, 1/h	0,04	0	
6	Cell grow rate, 1/h	0,2815	0,0905	
7	Direct toxicity, 1/h	0,3452	0,0668	
8	Metabolic toxicity, 1/h	0	0,1957	
9	Direct toxicity, %	100		

Figure 3 Input/output forms for Web-tool module (“TOX-SCREEN”)

Special excel template should be used to upload the input data to following calculations (**Figure 3)**. In order to save the results of simulations in form of *PNG file, the user should go to plot-panel (outlined in **Figure 2**) and the icon should be pressed.

2 “Sceen-toxicity” program. (Integrated screening model of cytotoxicity)

There is the base model for the estimation of biomaterial biocompatibility using in vitro tests. This service is expected to be the one most in demand. That is why it has a full-functional interface with the option to input the data of different experiments.

The model implements evaluation of biomaterial toxicity properties in vitro using toxicodynamic approach that take into account cell proliferation rate. This approach was specially developed for evaluation of biomaterials with low-toxic activity as it expected for new produced biocompatible materials for medical and biological applications. The program provides possibility to define toxicological profiles of biomaterials in terms for prediction of general toxicity pathways (direct, secondary, mixed). The service also has an option to take into account physiological deviations (fluctuation) in cell culture upon experimental period and the workflow of the application assumes automatic fitting of input values to avoid of modeling errors.

When the model is selected (“Screen-toxicity”) and program started, a corresponding part of the web - form will be outlined so the user will be able to choose the follow-up of the program (**Figure 4**).

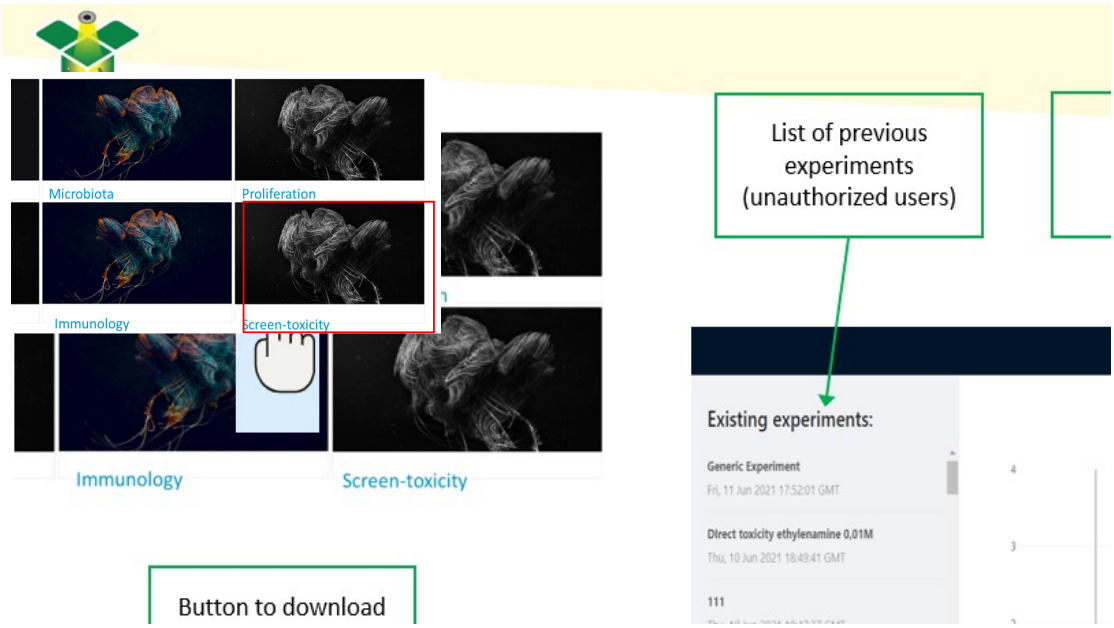


Figure 5. Input page of program “screen-toxicity”

User can look through and choose a data from the “List of existing experiments” or log in under own ID and continue to work with separate list of results. Otherwise, the user can continue in nonauthorised mode, but all the simulations fulfilled will be available for anybody who will enter the service.
The service also provides possibility simply let the application chose default values. End-user can change these values in the form (Table with uploaded values) in order to choose the desirable input data to estimate different possibilities (**Figure 5**). (Note: the web-page should be reloaded in this case preliminary to avoid errors). There is the button for upload the file in form of special excel file with experimental values that can be also used for data input.
End-user has an option to select two kinetic sets of values depend on a time – kinetic of cell viability under influence of an insult upon a time and kinetic set of the value in the corresponding control.
Once the parameters are set (end-user input or selection of default values) the calculation can be run. The finished calculation will return the results that can be downloaded.
The manual for visualisation of the results, as well as suggestion for analysis of the obtained results can be found by clicking on the button on the “login” web-page.

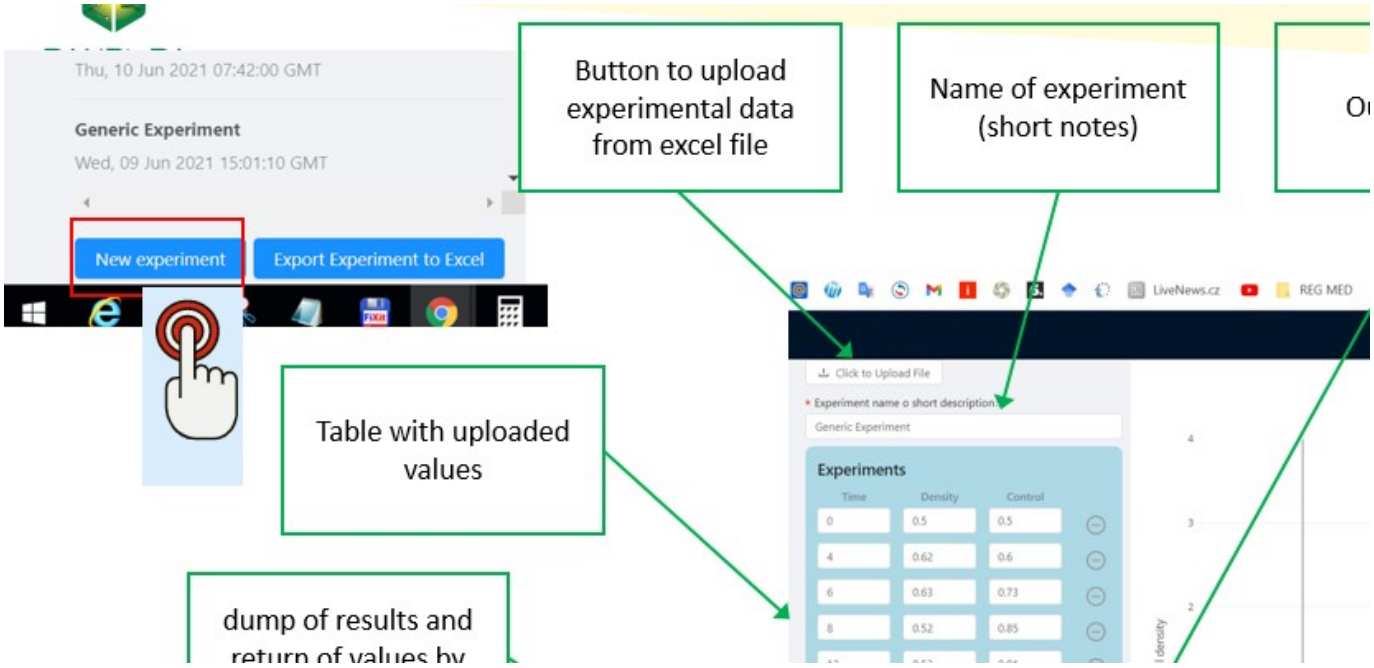


Figure 5. User interface of program “Screen-toxicity”.

The results visualization is performed on the platform itself, meaning that once the calculation is finished, the simulation results will be displayed on the page, in the form of a different images and numerical parameters. Each results will be stored in database and retrieved by the request. Results displayed in the form of images, represent the toxicodynamic process in cell culture in time. After the calculations are carried out, the results can be downloaded as .png image to a local computer or in the form of excel file.
The separate modeling set can be marked and downloaded using special brief note or according to exact time of simulation procedure from the list of “existing experiments”.
Example of simulation for insult with predominantly direct toxic effect (experiment name: Direct toxicity ethylenamine 0,01M, hepatocytes) outlined in **Figure 6**.

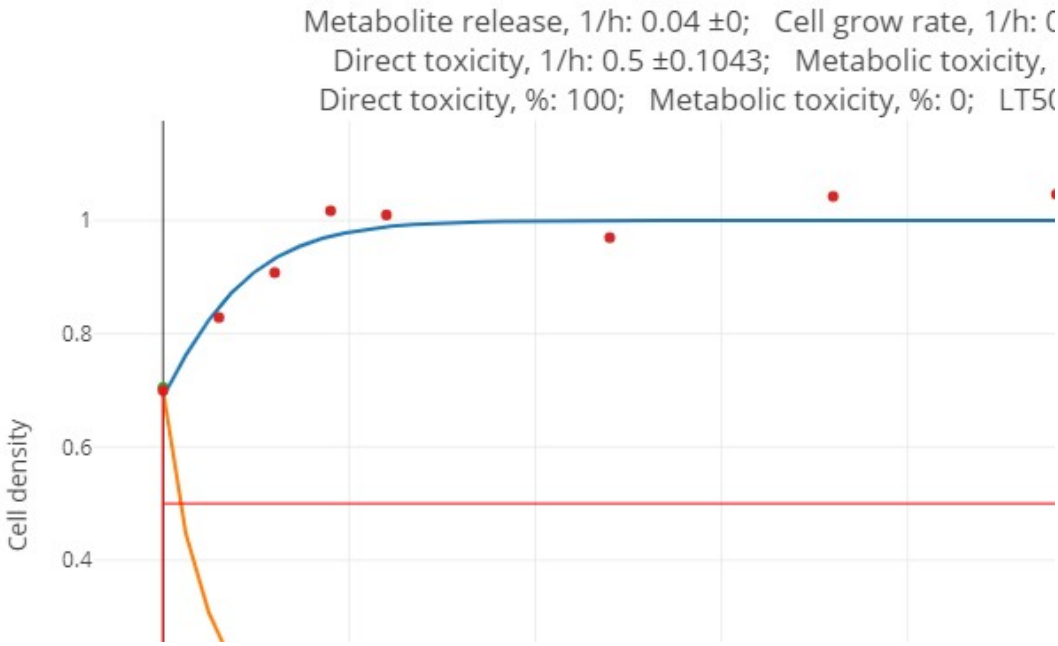


Figure 6. Results of simulation. Experiment: “Direct toxicity ethylenamine 0,01M, hepatocytes”

Example of simulation for insult with predominantly secondary (metabolite-associated) toxic effect (experiment name: 1mM acetaminophen - HEPATO cell indirect, hepatocytes) is outlined in the **Figure 7.**

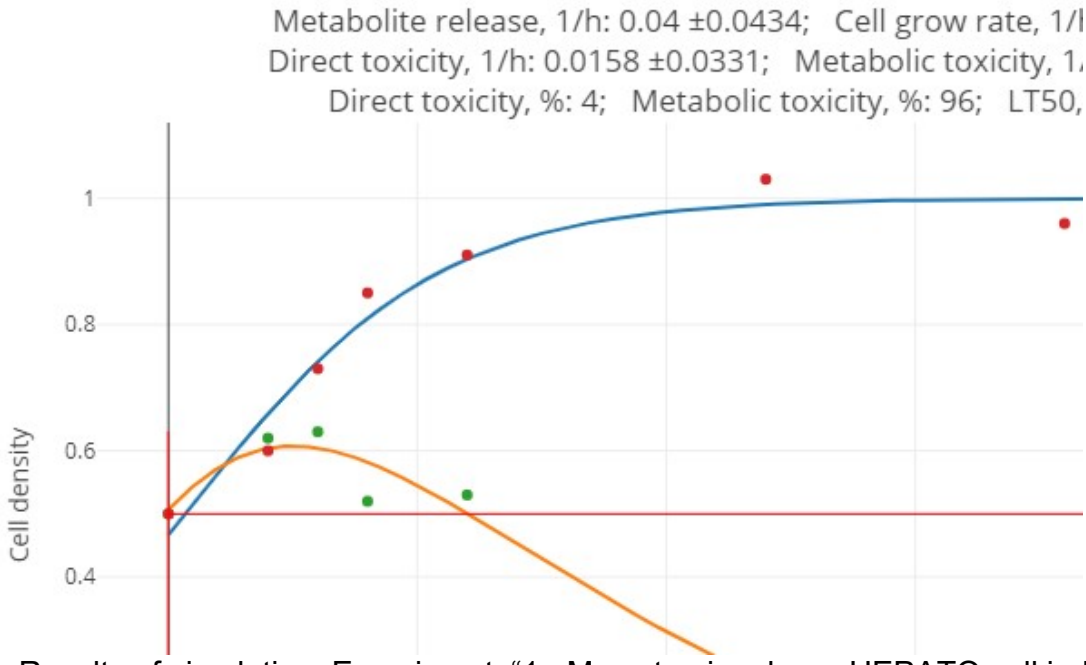


Figure 7. Results of simulation. Experiment: “1mM acetaminophen - HEPATO cell indirect, hepatocytes”

Some other additional options are available, viewing the value for any moment in time - both for input experimental data and fitted curve result, displaying the results of the plot fragment in an enlarged format, etc. This is achieved by using of additional plot-panel outlined by **Figure 2**.

Interpretation of 7 output parameters characterizing insult toxic properties in vitro according to investigation of cytotoxicity kinetic:

- “Cells growth rate” - rate of increase of cells population per unit of biomass
- “Metabolite release constant” - rate of increase of metabolite per unit of toxin.
- “Metabolite toxicity rate” – metabolite-associated (secondary toxic influences) rate of decrease of cell amount per unit of biomass and unit of metabolite
- “Direct toxicity rate” – rate of decrease of cell amount per unit of biomass and unit of initial toxin that cause direct toxic effect on cells.
- “Direct toxicity %” – relative statistical index outlined the input of direct toxical processes in cell destruction.
- “Metabolite toxicity %” - relative statistical index outlined the input of secondary (metabolite-associated) toxical processes in cell destruction.
- “LT50” – the semi-lethal time for insult in applied concentration per unit of cell.

3 Program “Immunology” (Model of immune response to antigen – adapted for soluble antigens and nano-objects).

The model for screening estimation of immune responce sequence and activity are used for the program development. The pro-inflammatory phase is characterised by M1 factors (e.g. IL-1) and macrophage activation, whereas anti-inflammatory phase followed with reciprocal effect and secretion of M2 factors (such as IL-10) is accompanied by attenuation of immune response
When the model is selected (“Immunology”) and program started, a corresponding part of the web - form will be outlined so the user will be able to choose the follow-up of the program (**Figure 8**).

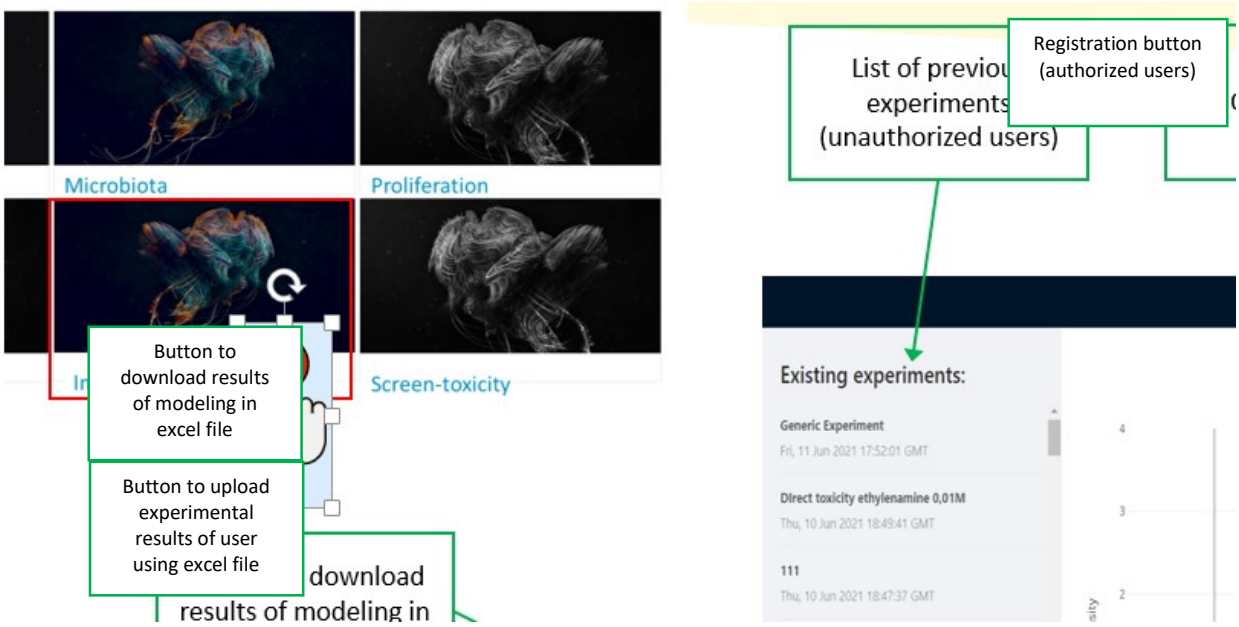


Figure 8. Input page of program “Immunology”

There is the button for upload the file in form of special excel file with experimental values that can be also used for data input. End-user has an option to select three kinetic sets of values depend on a time – kinetic parameters of macrophage activation, M1 – kinetic parameters and M2 - kinetic parameters. **Figure 9.**

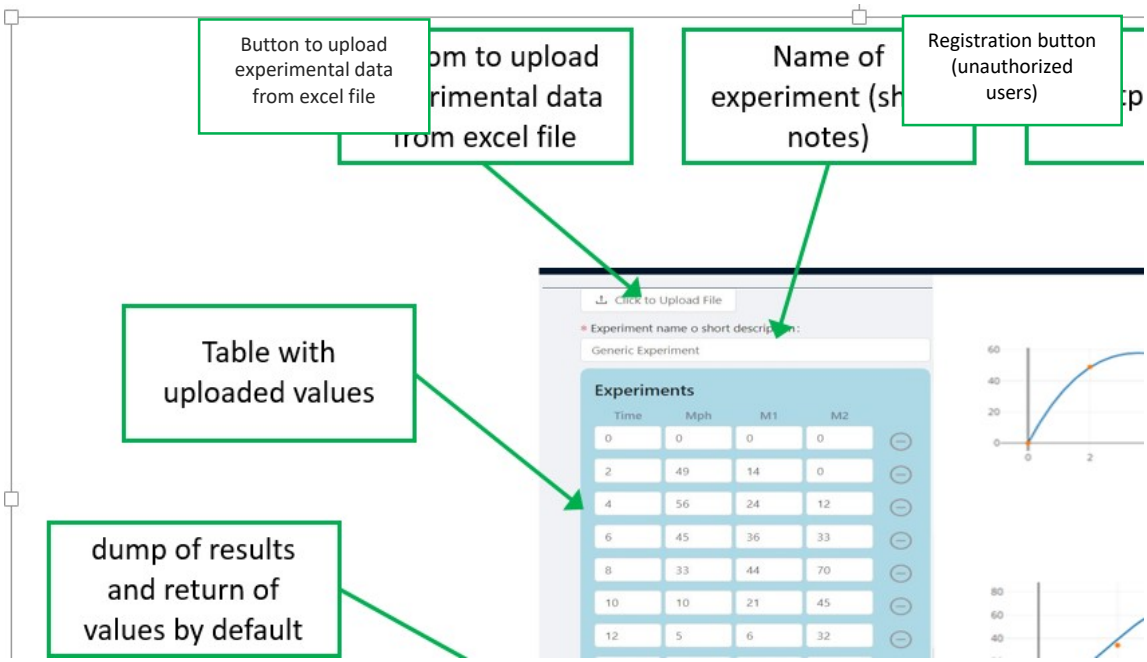


Figure 9. User interface of program “Immunology”.

Once the parameters are set (end-user input or selection of default values), the calculation can be run. The finished calculation will return the results that can be downloaded.

Each results will be stored in database and retrieved by the request. Results displayed in the form of images, represent the toxicodynamic process in cell culture in time. After the calculations are carried out, the results can be downloaded as .png image to a local computer or in the form of excel file.

The separate modeling set can be marked and downloaded using special brief note or according to exact time of simulation procedure from the “List of experiment”. Example of simulation for biomaterial, which have not induced excessive immune response (experiment name: “Normal immune response”) is outlined in the **Figure 10**. The resulting value are not exceed 1,1 ratio (Mph=1.01; M1(IL-1) =1.01;M2 (IL-10)).

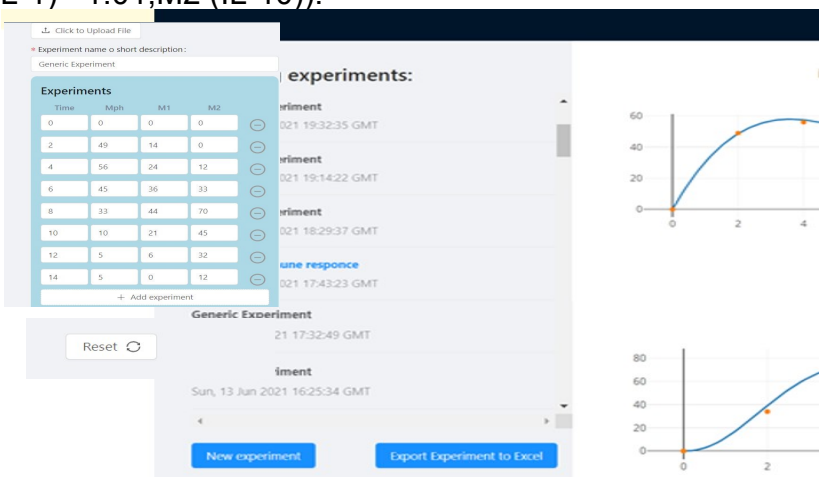


Figure 10. Result of simulation. Experiment: “Normal immune response; E. Kumolosasi (2014)”.

Example of simulation for biomaterial which induced excessive immune response (experiment name: Pathological immune response) is outlined in the **Figure 11**. At least the one of resulting value exceeds 1,1 ratio (Mph=1.01; M1(IL-1) =1.44;M2 (IL-10)).

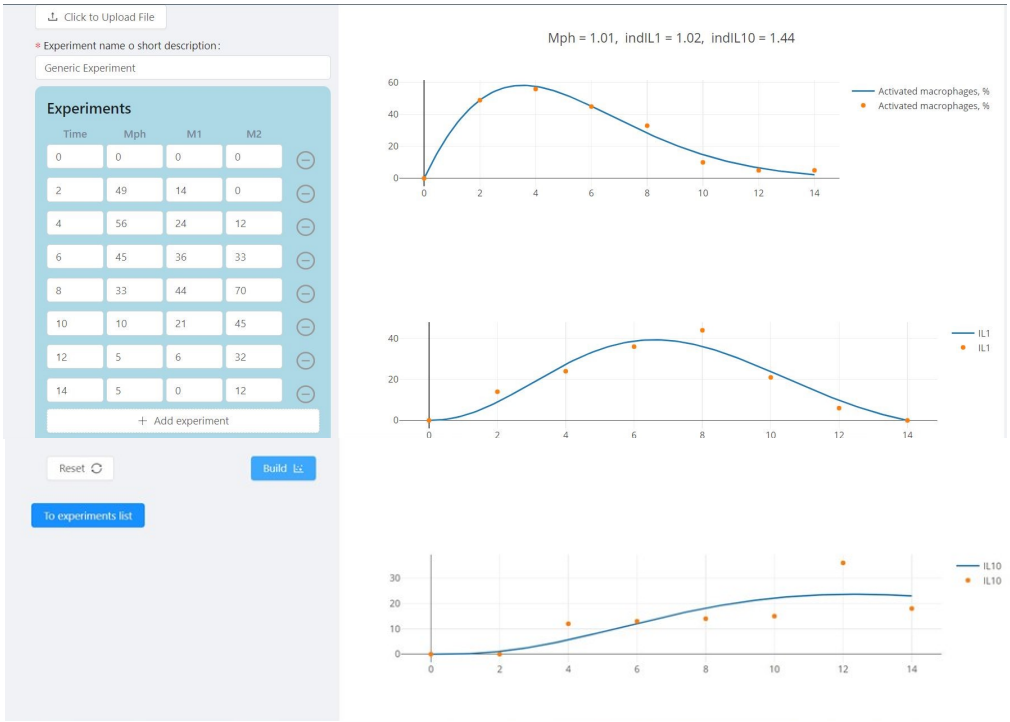


Figure 11. Result of simulation. Experiment: “Pathological immune response; E. Kumolosasi (2014)”

4 Program “Microbiota” (Model of microbiota-mammalian cell interaction under biomaterial influence).

The microbiota interactions service is designed for analysis and prediction of time evolution of an in vitro multispecies systems under influence of investigated insult/biomaterial. This model has been developed in order to characterize the microbial population dynamics in close interaction with mammalian (epithelial) cells resulting from competitive and mutualistic interactions and influence of biomaterials on these cell populations, as it occurs in living organism (mucosa compartments). This model gives an approximation of how the biomaterial can create an opportunistic environment for symbiotic/antagonistic interaction of different microbes (e.g. *S. Aureus*, *C. Albicans etc.*) and mammalian cells (epithelial). Definition of microorganism proliferation during a few generations gives possibility to solve the inverse problem - to define the level of biomaterial compatibility with normal microbiota.

When the model is selected (“Microbiota”) and program started, a corresponding part of the web - form will be outlined so the user will be able to choose the follow-up of the program (**Figure 12**).

In order to achieve an appropriate result of simulation user should provide kinetic experimental data of the 3 (2 as an option) different cell types development inside the bioreactor (denoted as “Pathol”, “Antag.”, and “E-cell”). By moving sliders in the upper-left corner of “User interface page” and enter initial cell densities of all 3 types on the “Init” lines of two tables below user has the possibility to

adjust parameters of microbiota interaction. It is possible also to fill the tables with measured cell densities from experiments at different times by manually changing the pre-existing values specified initially in the tables for illustrative purposes. After that “Build model” button should be pressed to start the program. Please take into account that the program fields cannot be filled with any values, as well as input parameters can be changed in certain limits only by sliders, otherwise a program error occurs. This is due to both a limitation defined by the program code and an inherent limitation inside the mathematical model itself. Two graphs on the right panel present time evolution of the model for two sets of initial conditions provided in the “Init” lines of two tables, higher and lower table corresponding to higher and lower graphs. By hovering mouse pointer close to the lines on the graph, the user can check exact model predictions for a given time, as it is demonstrated.

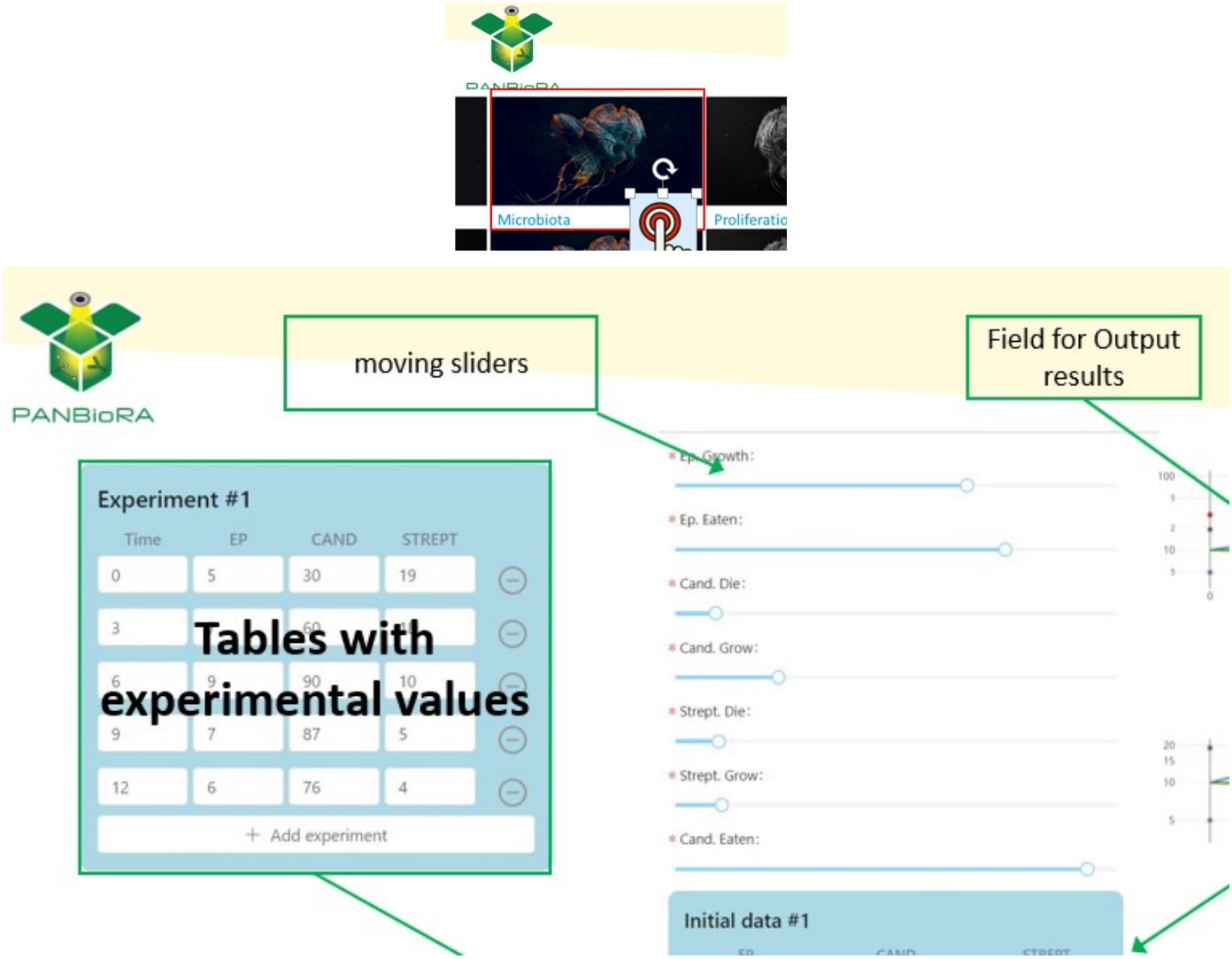


Figure 12. User interface page of the program “Microbiota”

End-user has an option to select three kinetic sets of values depending on time – kinetic of mammalian cell viability/proliferation, kinetic of pathogenic microbes viability/proliferation and kinetic set of the commensual microorganisms viability/proliferation. Two separate tables with different data sets should be filled (one for experimental set and other for control set (without biomaterial/insult).

Once the parameters are set (end-user input or selection of default values) the calculation can be run. The finished calculation will return the results that can be downloaded.
The results visualization is performed on the platform itself, meaning that once the calculation is finished, the simulation results will be displayed on the page, in the form of a different images and numerical parameters. Each results will be stored in database and retrieved by the request. Results displayed in the form of images, represent the process of different cell kinds' interaction in cell culture during cultivation. After the calculations are carried out, the results can be downloaded as .png image to a local computer or in the form of excel file. The parameter "Chi" shows the statistical significance of the level of deviation of the studied parameters from the median dependence between the data series (for each type of cells). At the same time, Δ (fitted parameter value) reflects the dependence of the development of pathological microorganisms from the influence of biomaterials on the cell system as a whole.

Example of calculation is presented below **Figure 13.** On the graphs presented mammalian (epithelial) cells are denoted by blue curve, pathogenic (opportunistic) microbes by brown curve, mutualistic bacteries as green curve. First plot represents the situation when streptococci had been suppressed by antibiotics so that candida start to grow and suppress epithelium substantially. Second plot show more or less equilibrium dynamics of all three cell types.

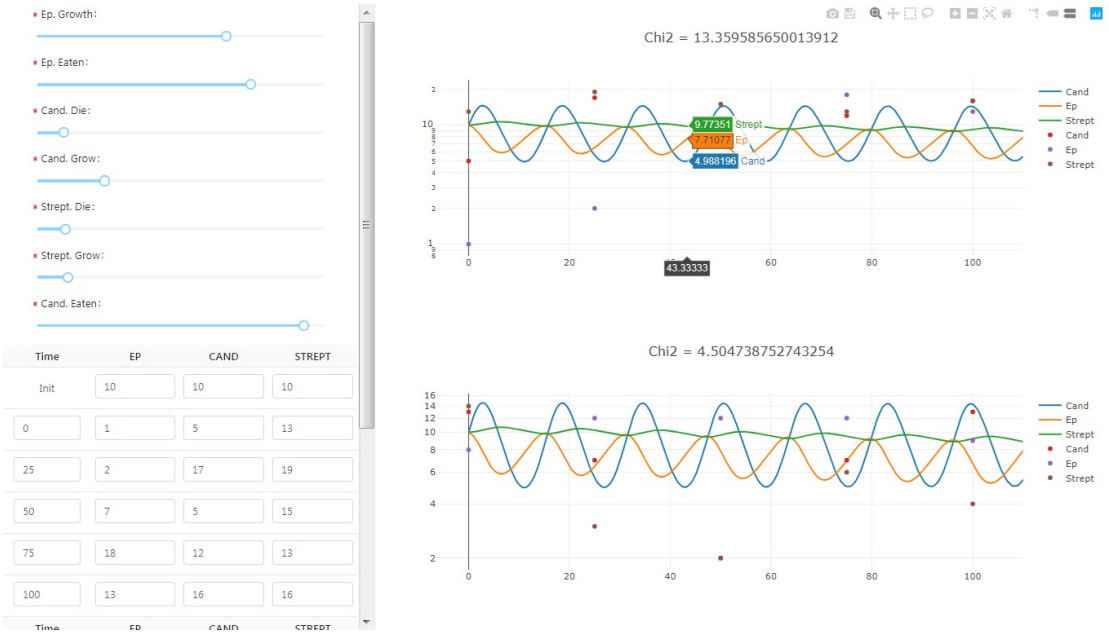


Figure 13. Result of simulation. Experiment: "Candida-streptococci".

Other example of simulation for unstructured gelatine influence on Staphylococcus Aureus/HACAT coculture is outlined in the **Figure 14**. This represents experimental findings concerning simpler two-species microbiota model of keratinocytes (HACAT) and Staphylococcus Aureus interaction. The mutual influence of every cell population with/without non-structured gelatin as a substrate described here on the competition between bacteria and epithelial cells demonstrated that the mammalian cells rapidly loose the viability and detach from the surface in the presence of high concentration of microbes.

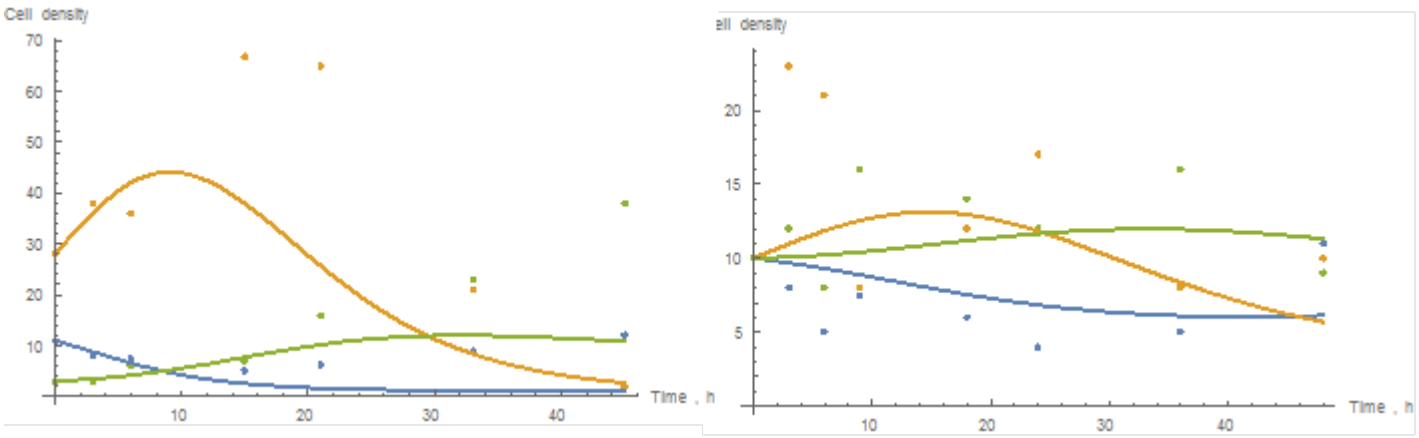


Figure 14. Result of simulation before fitting. HACAT viability inhibition.

The separate modeling set can be marked and downloaded using special breaf note or according to exact time of simulation procedure from the “List of experiment”.

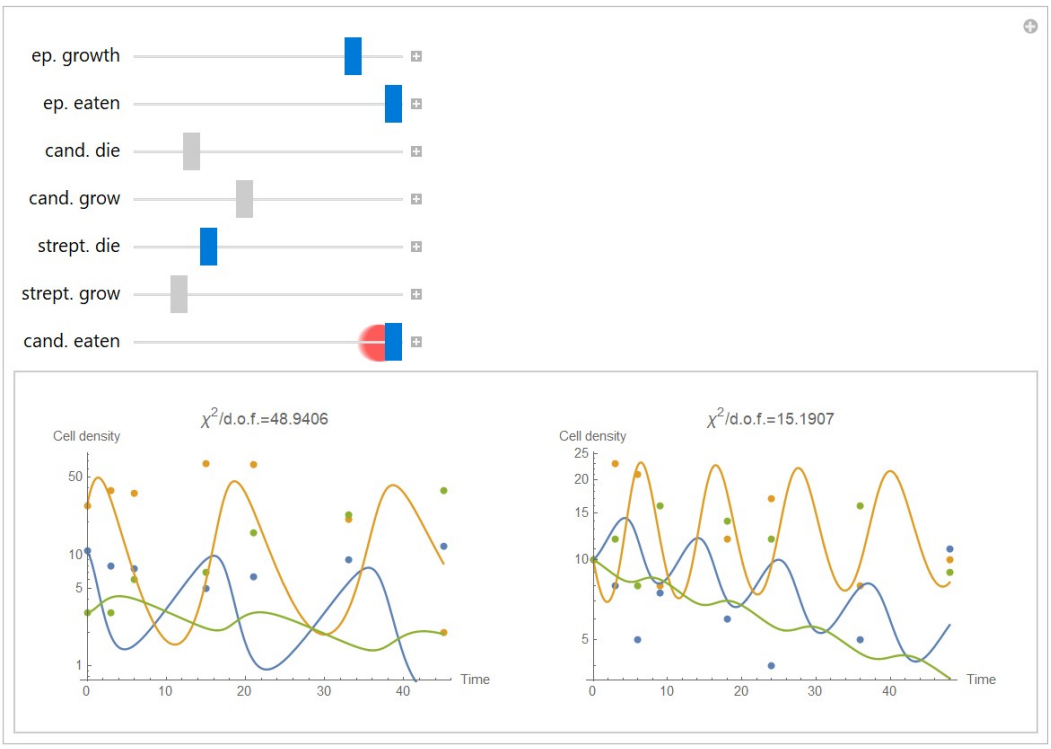


Figure 14. Result of simulation after fitting using the program “microbiota”. HACAT viability inhibition.

5 Program “proliferation”. (Discriminative analysis of proliferation kinetics).

Proliferation process is essentially stochastic and as was shown by experiments of our collaborators, measurements of the cell density are quite noisy, so the method cannot rely on the fitting of experimental data to a solution of the differential equation by itself—due to the memory of the process starting from a small stochastic deviation experimental curve will deviate more and more from the computed one with the course of time. Instead, the relative proliferation rate, however noisy, does not have the memory effect and can be used to discriminate toxic substances from control.

To detect the toxic effect we use least squares method applied to the relative proliferation rate (dn/dt)/n as a function of the cell density n, that should be represented by a straight line in the proliferation model adopted. From the parameters of the line the base proliferation rate and the limiting cell density are derived along with their uncertainties, and then they are compared between experiment and control to check whether any of them for the substance under the test is substantially lower than control (**Figure 15)**.

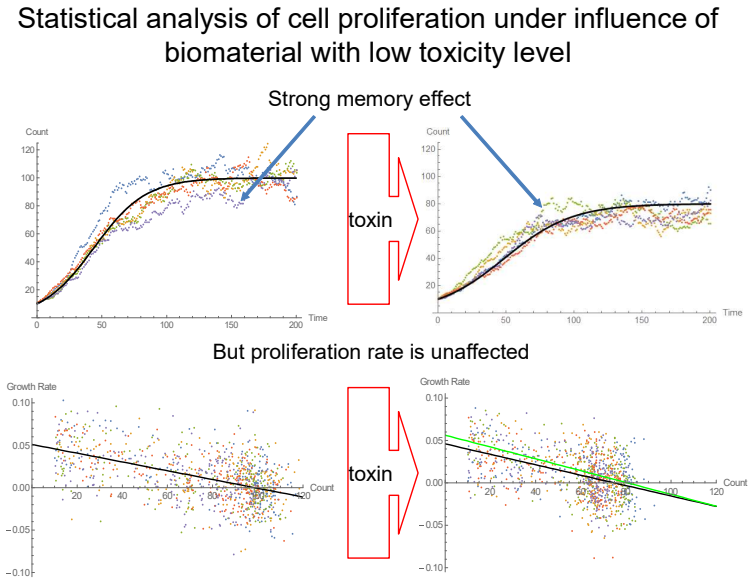


Figure 15. Statistical analysis of cell proliferation under influence of biomaterial with low toxicity level.

Start page of service has the form shown in Figure below. User should fill the tables with measured cell densities from control (top form) and experiment (lower form) at different times manually changing the preexisting values specified initially in the tables for illustrative purposes. After that “Build model” button should be pressed to start the program. Please take into account that the program fields cannot be filled with any values, otherwise a program error occurs as explained before.

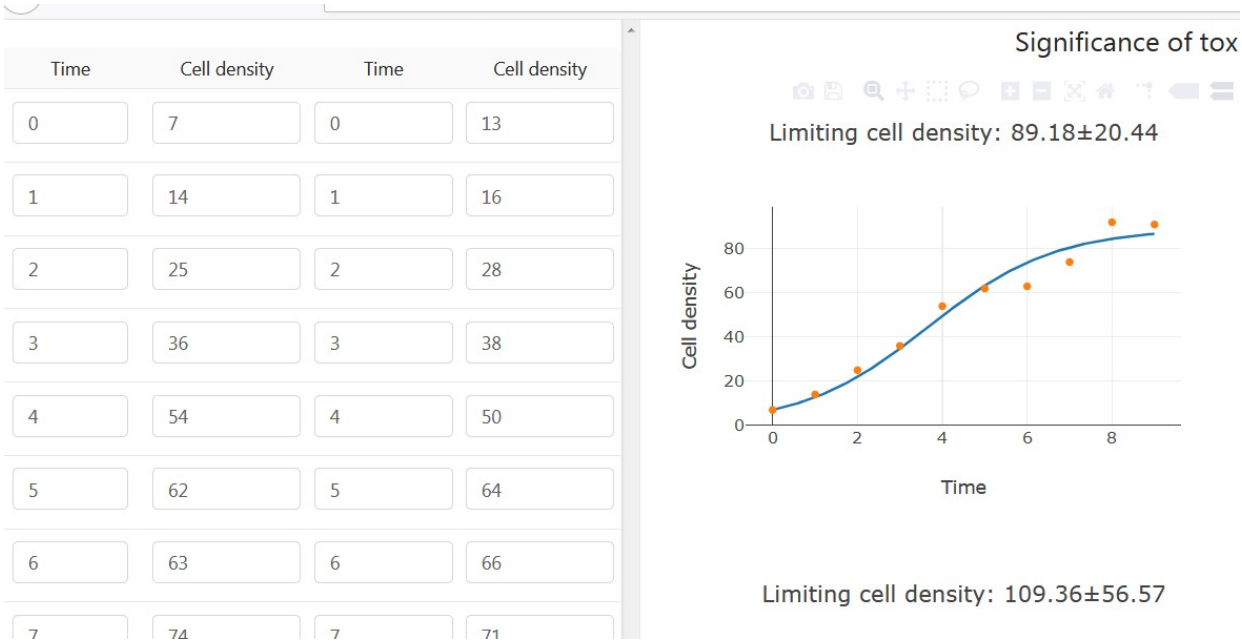
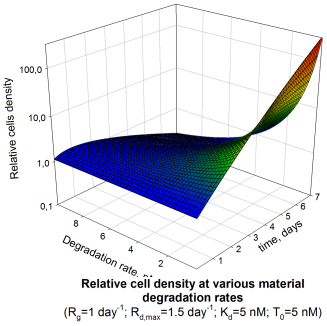
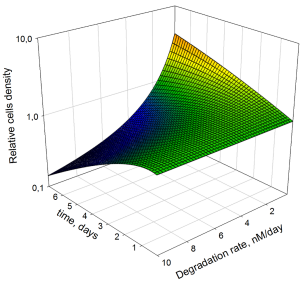


Figure 16. Interface page of “Model of discriminative analysis of proliferation kinetic

Example of calculation according to experimental data is presented in the Figure above. The analysis reveals the 2-time lower base proliferation rate for tested biomaterial.
In the example shown toxic effect is profound (experimental base proliferation rate is significantly lower than control one), its significance is so close to 1, that the applied algorithm for estimation of significance produce value higher than 1 due to limitations of machine precision.

6 Program “fit-experiment”





Biodegradation rate of the toxic material is the property of utmost importance for the cell proliferation. If the rate is small enough and toxin is not present in the system at the initial moment, the toxic effect will be masked at the beginning, and can be recovered from long-run observation only. You can see it on the left graph – for the toxicity parameters chosen cell density starts to grow similarly for any degradation rate, and start to deviate only at late times. This effect can be partially alleviated by the initial presence of toxin in the system, as you can see on the lower graph.

When the toxin is continuously released from the biomaterial at a constant rate k [nM / day] and acting on the cells causes their proliferation or death, the concentration of toxin is not constant.

The program represent simplified calculator, which operate with initially known value. The main purpose of this program is to define the most appropriate diapason of concentration (insult, cells) for further development of optimal experimental design **Figure 17.**.

7 Program “hep-toxicity”

The program represent reduced model of cytotoxicity adapted for preliminary estimation (prediction) of hepatotoxicity (LT50 only) of insults when its concentration is constant, and mechanisms of cell damage is unknown.

Specific for a cytotoxicity experiment in which the concentration of the toxin will remain constant during the test. If cell proliferation is taken as neglected, then in the first approximation the rate of cell death can be considered proportional to the product of the concentration of target cells, the concentration of insult and a parameter characterizing the toxic properties, for example, the rate of degradation of the polymeric material. **Figure 18**.

Figure 18. Prediction of biomaterial hepato (cyto-) toxicity.